the spinal cord injury and restoration of spinal cord function. It is particularly unexpected given the complexity of the *in vivo* injury that the present invention would result in an increase in nerve function and behavioral recovery, evidencing that the present method may effectively be used to treat trauma patients and other patients having suffered from spinal cord injury. It is respectfully submitted that the Examiner has not cited any art which rises to a level of expectation that polyalkyelene glycols may be used *in vivo* in a method of treating a trauma patient or other mammalian patient which has suffered a spinal cord injury. In support of the patentability of the present invention, Applicants submit the declaration of Dr. Richard B. Borgens, co-inventor of the subject matter of the present application. It is respectfully submitted that the instant claims are in condition for allowance and such action is earnestly solicited. Support for the amendment to the claims can be found throughout the originally filed specification and claims and in particular, at pages 12, bottom paragraph and 15, first section, and in the examples, where the behavioral recovery of the laboratory test animals is exhibited. No new matter has been added by way of this amendment.

The Examiner has variously rejected originally filed claims 1-21 under 35 U.S.C. §112, first and second paragraphs and §103. For the reasons which are set forth in detail hereinbelow, Applicants respectfully submit that the instant invention is patentable.

The §112, First and Second Paragraph Rejections

The Examiner has rejected claims 1-8 and 11 under 35 U.S.C. §112, first paragraphs for the reasons which have been stated in the February 27, 2001 office action. In order to expedite allowance of this application and obviate the Examiner's rejection, Applicants have amended the claims to reflect the fact that C_1-C_{10} polyalkylene glycols are now claimed. Notwithstandings this amendment, Applicants respectfully submit that the claims embrace logical extensions and equivalents of the claimed polyalkylene glycols. It is respectfully submitted that the claims are now in compliance with 35 U.S.C. §112, first paragraph.

The Examiner has also rejected originally filed claims 6-8 as being indefinite under 35 U.S.C. §112, second paragraph for the reasons which are stated in the office action. Inasmuch as Applicants have cancelled claims 6-8 in the present amendment, the Examiner's §112, second paragraph rejection of claims 6-8 is now moot.

Applicants respectfully submit that new claims 22-43 are now in compliance with the requirements of 35 U.S.C. §112.

The §103 Rejection

The Examiner has rejected original claims 1-15 under 35 U.S.C. §103 as being unpatentable over Bittner, et al. ("Bittner", *Brain Research*, 367, 351-355, 1986), Krause and Bittner, et al., ("Krause I", *Proc. Natl. Acad. Sci. USA*, 87, 1471-75, 1990) and Krause, et al., ("Krause II", *Brain Research*, 561, 350-353, 1991), in view of Potter, et al., ("Potter", *Clin. Invest. Med.*, 19(4) Suppl.: S80 #533) and Ishikawa (WO97/35577, equivalent to as United States Patent Number 6,090,823).

The Examiner cites Bittner for teaching the use of PEG to join a severed axon of a mammalian cell line. Krause I is cited by the Examiner for teaching the direct application of a predetermined concentration and molecular weight of polyethylene glycol (PEG) to restore the morphological continuity of severed axons. Krause II is cited by the Examiner for teaching the direct application of a predetermined concentration and molecular weight of PEG to restore the functional continuity of a crushed axon. The Examiner argues that the references suggest that the application of PEG may be used for the reconnection and functional recovery of severed and crushed myelinated mammalian axons.

The Examiner recognizes that none of the above-cited references cites the use of 4-

aminopyridine for treatment of spinal cord injury. The Examiner cites Potter for teaching the use of 4-aminopyridine to treat spinal cord injury and cites Ishikawa for providing further motivation for using PEG in spinal cord injury treatment compositions. From the above-described references, the Examiner argues that the present invention is obvious and therefore, unpatentable. Applicants respectfully traverse the Examiner's rejection.

The present invention, as set forth in new claims 22-43 is directed to a method for treating a mammalian patient having suffered a spinal cord injury to its spinal cord, the method comprising contacting the injured spinal cord as soon as is possible and within a period of no greater than about 24 hours after the injury with an effective amount of an alkylene glycol as claimed, the method resulting in at least partial restoration of nerve function in the injured spinal cord and an increased reflex behavior in the patient after the spinal cord is treated. It is respectfully submitted that the present invention, which is directed to actual treatment of patients who have suffered spinal cord injury with a resulting favorable *unexpected* result, represents an invention clearly patentable over the art of record. For the reasons which are set forth hereinbelow, it is respectfully submitted that the present invention is clearly patentable.

The Examiner has cited Bittner, Krause I and Krause against the present invention. Each of these references is clearly deficient in failing to render the present invention unpatentable, either alone or in combination with the other cited art. Bittner is directed to *in vitro* experiments involving the reconnection of severed nerve axons (axoplasmic fusion) in *crayfish*. In addition to conducting experiments in crayfish, Bittner also conducted experiments wherein severed axon-like processes of a mammalian neuroblastoma/glioma cell line *seemed* to be joined to the cell body using PEG in tissue culture. In Bittner, the results of axoplasmic fusion were somewhat mixed, with only certain of the axons producing axonic continuity or, in certain instances, the complete removal of nuclear, axoplasmic and glial plasma membranes. In addition, Bittner indicates that partial axonic collapse and thickening

of the glial membranes was seen even in the cases of successful axoplasmic fusion. Even in the successful experiments (p. 353, column 1) using crayfish axons, only one of four neurons actually had an ability to conduct action potentials through a lesion site.

In other experiments involving *mammalian* axonic processes, the results set forth in Bittner were actually rather disappointing (p. 353, bottom of first column and second column) and *teach away* from the present invention. In the case of severed axonal processes in NG 108-15 hybrid cells derived by fusion of a mouse neuroblastoma clone with a rate glioma clone, the Bittner experiments proved disappointing, with a number of axons evidencing degeneration (p. 353, column two, top). As Bittner states on p. 353, "Because of the extensive number of processes in tissue culture and the fact that we could not pull severed processes apart before re-approximating them, we cannot be as certain as with MGAs that PEG produced morphological and functional fusion of completely severed axon." It is noted here that the disappointing Bittner results occurred in *in vitro* experiments under rather exceptionally favorable conditions, with experimenters attempting to line up and reapproximate the severed axonal ends. In a final experiment described by Bittner, scientists attempted to fuse severed axons from rat sciatic nerves with cat spinal cord. In no case was there demonstrated axonal fusion. (Bittner, p. 353, column 2, bottom).
Thus, despite the favorable conditions under which Bittner conducted his experiments, it is respectfully submitted that the Bittner results in *mammalian cells* actually suggest that the present invention could not readily occur. It is respectfully submitted that the disclosure of Bittner actually *teaches away* from the present invention, given the disappointing results Bittner discloses for experiments conducted with mammalian axons in an *in vitro* setting.

Turning to the disclosure of Krause I, it is respectfully submitted that this reference in no way obviates the clear deficiencies of Bittner in failing to disclose or suggest the present invention. Krause I discloses a series of experiments involving severed myelinated axons from the earthworm. In the disclosed experiments, under *in vitro* conditions, polyethylene

glycol was used to morphologically repair the cut ends of invertebrate myelinated central nervous system axons in the earthworm. It is noted that Krause I chose to utilize the earthworm in the disclosed studies because of the nature of the earthworm and its axonal (medial giant axon or MGA) processes. In addition, the earthworm MGA exhibits a natural ability to fuse during development which was postulated to render the MGA more susceptible to PEG fusion. In the Krause I experiments, although a return of morphological function to the severed axons was significant, there was some evidence of an ability to conduct a current after repair (using lucifer yellow as an indicator), but absolutely no evidence that the method would produce any further functional increase even in an *in vitro* setting. Although Krause I does postulate the *possibility* that the method might be used in vertebrates, the disclosure is riddled with uncertainty and conjecture and Krause provides absolutely no evidence for such a conclusion. Moreover, the suggestion that the method may be used in circumstances where the severed axons could be carefully aligned in a nerve bundle (p. 1474, second column, bottom), indicates that Krause I doubts the practicality of such an approach. It is respectfully submitted that Krause I does nothing to obviate the deficiencies of Bittner in failing to teach the present invention.

Turning to Krause II, this reference, like Krause I, does nothing to cure the deficiencies in the art in producing the present method. Krause I is directed to the use of PEG to artificially restore electrical continuity across a crushed lesion of a medial giant axon (MGA) of the earthworm. As in Krause I, Krause II provides further *in vitro* evidence in an invertebrate model. However, Krause II, like Krause I, provides absolutely no evidence that the present method may be performed *in vivo* to treat mammalian patients.

In contrast to the cited art, the present invention has evidenced unexpected activity in restoring nerve function and behavioral recovery in mammalian patients. The present invention could not possibly be predicted from the prior art and represents an unexpected result over the disclosure of the prior art. In the present invention, as described in the

examples of the specification, a dramatic response to treatment with PEG was realized in experimental animals (guinea pigs). In the present invention 100% of experimental animals treated with PEG evidenced substantial return of cord conduction vs. 0% of controls and 90% PEG-treated guineas pigs exhibited a return of behavior vs. 17% of controls. These results represent an unexpected result and evidence that the present method exhibits great potential to treat patient who have suffered spinal cord injury. The prior art *in vitro* methods could not possibly provide any level of expectation which might approximate the *in vivo* treatment. This is because there are simply too many factors which could influence treatment in the *in vivo* treatment as compared to the rather simple and controlled *in vitro* experiments of the art. A brief recitation of these differences is summarized below.

1. In the *in vitro* experiments, in the chamber which is used for the experiments, there is no blood supply to the cord. The blood is washed completely out of the anaesthetized animal prior to the dissection of its spinal cord. In stark contrast, in the animals treated by the present invention, hemorrhagic injury is a hallmark of spinal cord injury. In fact, spinal cord damage is clinically referred to as "Central Hemorrhagic Necrosis" after Professor Reginald Allen (1910). Note that there are issues of swelling and blood loss (ischemia) but also numerous biochemical consequences of blood chemicals (heme from hemoglobin) which catalyze various destructive chemical reactions which lead to death. None of these complex interactions occurs in the chamber used in the *in vitro* experiments of the art.

2. The dissected spinal cord within the isolation chamber of the prior art has had all of its nerve roots cut, and does not possess the brain which is left in the animal after dissection. There is absolutely no factual basis upon which one can predict behavioral consequences of conduction changes in the isolation chamber.

3. In the isolation chamber of the *in vitro* experiments of the prior art, many important physical processes are under strict control, such as oxygenation of the tissue, ionic concentrations in the perfusate, glucose concentration, temperature, etc. In contrast, in the living animal, all of these things are totally deranged and out of control following spinal trauma. Nothing is under control in the traumatized animal.

4. In the chamber, the isolated spinal cord ventral white matter is only a fragment of the nervous system. This fragment contains long expanses of single axons where one tests conduction. There are no synapses with other nerve cells in the tested "circuit" - just pure axon bundles kept alive under heroic and artifical conditions. In the living animal *behavior recovery* from spinal cord injury is a function of the entire neural circuit including millions of chemical synapses and the participation of innumerable other nerve and non-neural cells.

5. The dosage of drugs at the tissue injury site (related to turnover, metabolism, etc.) and their activity may be largely unknown in *in vivo* conditions, especially where introduction of the agent may be through and around tissue which may be hard to reach. In the present invention, *in vivo* administration is shown to be effective for treating spinal cord injury.

6. In the chamber, *in vitro*, the health of the animal is not a consideration in determining the end point defined as a "recovery". In the spinal injured animal, every fact of the animal's state of health participates in the recovery process or the lack of it.

By all measures, the present invention represents a significant advance over the art, which is directed to the use of PEG to provide essentially mechanical continuity to severed

axons. Nowhere in the prior art is there any evidence which would lead one of ordinary skill to conclude that the present invention could be performed with such measure of success in an *in vivo* setting. It is respectfully submitted that the *in vitro* experimentation provided no expectation of success in the *in vivo* animal, especially given the mixed results which were reported for the *in vitro* experimentation in the art cited references.

In further support of the present invention, the declaration of Dr. Richard B. Borgens is attached hereto. Dr. Borgens, a co-inventor of the subject matter of the instant invention, is professor of Neuroscience in the Department of Basic Medical Sciences, School of Veterinary Medicine, Purdue University. It is his opinion, as set forth in his declaration, that the instant invention is non-obvious over the prior art and the prior art represents a series of *in vitro* experiments which provide no basis upon which to predict that the present invention will have applicability in an *in vivo* setting. It is respectfully submitted that the instant invention is patentable over the art of record which at best, in combination, teaches that it may be *obvious to try* the present invention, with no expectation for success given the limitations of the vertebrate *in vitro* experiments in the art and at worst, the prior art *teaches away* from the present invention. Consequently, it is respectfully submitted that the present invention is patentable over the cited prior art.

Turning now to the Examiner's rejection of the claims which utilize a potassium channel blocker in combination with an polyalkylene glycol to synergistically treat spinal cord injury, it is respectfully submitted that the disclosures of Potter and/or Ishikawa and the disclosures of any one or more of Bittner, Krause I and Krause II fail to render those claims obvious. As discussed hereinabove, the present invention makes use of polyalkylene glycol compounds to treat spinal cord injury in mammalian patients. As discussed, none of Bittner, Krause I or Krause II in any way renders the present invention obvious. The disclosures of Potter and Ishikawa are essentially inapposite to the present invention inasmuch as these references do not in any way cure the deficiencies of the prior art. Potter teaches the use of

4-aminpyridine in spinal cord injuries but fails to even mention polyalkylene glycol. In the case of Ishikawa, this reference merely mentions polypropylene glycol and polyethylene glycol *in passing* as possible solvents for the active agent (nicaraven) which is used. Neither of these references recognizes the unexpected properties the polyalkylene glycols exhibit in treating spinal cord injury. Neither of these references even mentions polyalkylene glycols as a possible treatment for spin cord injury. Because of the deficient disclosures of Potter and Ishakawa, it is respectfully submitted that the claimed invention is patentable over these references.

As further evidence of the non-obviousness of the present invention, it is noted that the Examiner, in making the obviousness rejection, has had to cobble together three references, Bittner, Krause I and Krause II, which are all approaching ten years in age. Given the importance of the present invention, and its relevance to meeting a long-felt need in the art, one would have assumed that if the present invention was obvious over the prior art, the Examiner would have been able to cite a more relevant contemporary reference. It is clear from this deficiency in the art alone, that the art did not expect or contemplate the present invention. The present invention is patentable over the art of record.

For the above reasons, Applicants respectfully assert that the claims set forth in the amendment to the application of the present invention are now in compliance with 35 U.S.C. Applicants respectfully submit that the present application is now in condition for allowance and such action is earnestly solicited.

Applicants have cancelled 23 claims (two independent) and added 23 claims (two independent). No fee is therefore due for the presentation of this amendment. A petition for a three month extension of time is enclosed. Please charge any fee due to Deposit Account No. 04-0838.

Please note that the correspondence address for this application has changed tothat set forth below. A separate CHANGE OF CORRESPONDENCE ADDRESS is enclosed herewith.

Respectfully submitted,

Dated: August 27, 2001

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: "Commissioner for Patents, Washington, D.C. 20231" on August 27, 2001.

R Neil Sudol

R. Neil Sudol, Reg. 31,669

Appendix

22. A method of treating a mammalian patient having suffered an injury to its spinal cord, said method comprising contacting said spinal cord as soon as is possible and within a period no greater than about 24 hours after said injury with an effective amount of a C_1-C_{10} polyalkylene glycol, said method resulting in at least partial restoration of nerve function in said injured spinal cord and an increased reflex behavior after said spinal cord is treated.

23. The method according to claim 22 wherein said spinal cord is severed.

24. The method according to claim 22 wherein said spinal cord is crushed spinal cord.

25. The method according to claim 1 wherein said polyalkylene glycol is selected from the group consisting of polymethylene glycol, polyethylene glycol, polypropylene glycol, polybutylene glycol, polypentylene glycol, polyhexylene glycol, polyheptylene glycol, polyoctylene glycol, polynonylene glycol, polydecylene glycol and mixtures, thereof.

26. The method according to claim 25 wherein said polyalkylene glycol is administered to said patient in a pharmaceutically acceptable carrier.

27. The method according to claim 26 wherein said polyalkylene glycol is selected from the group consisting of polyethylene glycol, polypropylene glycol and mixtures thereof.

28. The method according to claim 22 wherein said polyalkylene glycol is

polyethylene glycol.

29. The method according to claim 26 wherein said polyalkylene glycol is polyethylene glycol having a molecular weight ranging from about 40 daltons to about 3500 daltons.

30. The method according to claim 22, wherein said method further comprises the step of contacting injured spinal cord with an effective amount of a potassium channel blocker before, during or after contacting said spinal cord with said polyalkylene glycol, said method resulting in a synergistic increase in restoration of nerve function and reflex behavior in said patient.

31. The method according to claim 30 wherein said polyalkylene glycol is selected from the group consisting of polyethylene glycol, polypropylene glycol and mixtures thereof.

32. The method according to claim 30 wherein said potassium channel blocker is an amino-substituted pyridine compound.

33. The method according to claim 31 wherein said potassium channel blocker is 4-amino pyridine.

34. The method according to claim 30 wherein said polyalkylene glycol is polyethylene glycol.

35. The method according to claim 32 wherein said polyalkylene glycol is polyethylene glycol having a molecular weight ranging from about 40 daltons to about 3500 daltons.

36. The method according to claim 33 wherein said polyalkylene glycol is polyethylene glycol having a molecular weight ranging from about 40 daltons to about 3500 daltons.

37. The method according to claim 34 where in said polyethylene glycol has a molecular weight ranging from about 40 daltons to about 3500 daltons.

37. A method of treating a mammalian patient having suffered an injury to its spinal cord, said method comprising contacting said spinal cord as soon as is possible and within a period no greater than about 24 hours after said injury with an effective amount of polyethylene glycol, said method resulting in at least partial restoration of nerve function in said injured spinal cord and an increased reflex behavior after said spinal cord is treated.

39. The method according to claim 38 wherein said polyethylene glycol has a molecular weight ranging from about 40 daltons to about 3500 daltons.

40. The method according to claim 38 further comprising the step of contact said injured spinal cord with an effective amount of a potassium channel blocker before, during or after contacting said spinal cord with said polyethylene glycol.

41. The method according to claim 40 wherein said potassium channel blocker is an amino-substituted pyridine.

42. The method according to claim 40 wherein said potassium channel blocker is 4-aminopyridine.

42. The method according to claim 42 wherein said polyetheylene glycol has a molecular weight ranging from about 40 daltons to about 3500 daltons.